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## Original Article

# Effects of increased begging and vitamin E supplements on oxidative stress and fledging probability

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The evolution of conspicuous begging displays has been suggested as the outcome of a conflict where offspring attempt to manipulate food allocation beyond the parental optimum. One resolution for the conflict arises via costs of begging, and oxidative stress has been proposed as a major mechanism for causing begging-induced costs. Although begging can be a physically demanding activity, the evidence for causing oxidative stress is scarce. Great tit (*Parus major*) parents provide food at the nest mostly from 2 different locations, which in consequence relaxes nestling competition. Here, we manipulated nestling competition by forcing parents to feed from a single location and supplemented half of the nestlings in each brood with vitamin E to test if this major antioxidant can alleviate a potential oxidative cost of begging. The design increases the cost of begging without altering parental feeding rates. Begging intensity was significantly higher when parents fed from a single location. Body mass and antioxidant capacity were not affected by the increase in begging, but oxidative damage was lower in females of the increased begging group compared with those in the control group, independent of vitamin E supplementation. The results suggest that oxidative stress is rather a minor cost of begging. Vitamin E-supplemented nestlings had a higher probability to fledge, which underlines the important role of vitamin E during development, although this might not be due to its role as an antioxidant.

**Key words:** begging, oxidative stress, parent–offspring conflict, *Parus major*, vitamin E.

## INTRODUCTION

The parent–offspring conflict emerges from the fact that parents and their offspring have different genetic interests regarding the allocation of resources (Trivers 1974). The young of many species use conspicuous begging displays to demand resources, in particular food, from their parents. Several theoretical models have been developed on the evolution of begging (Harper 1986; Grafen 1990; Godfray 1995; Rodríguez-Gironés et al. 1996; Parker et al. 2002). In order to maintain begging on an evolutionary stable level, almost all models imply that begging should not only entail benefits but also costs (Mock and Parker 1997). Different kinds of costs have been proposed, and some of them have been tested experimentally (see Moreno-Rueda 2007, and references therein). Empirical evidence and the underlying physiological mechanisms for a cost of begging remain yet equivocal.

Oxidative stress has been suggested as a possible cost of begging behavior (Costantini et al. 2006; Moreno-Rueda 2007; Noguera et al. 2010). Bird begging behavior usually encompasses physically

demanding activities like posturing, wing flapping, and stretching (Kölliker et al. 1998), which are expected to rise the production of reactive oxygen species (ROS). When an individual is not able to outweigh ROS with its antioxidant defense system, oxidative stress occurs (Sies 1991). Oxidative stress can cause damage to tissues and, therefore, lead to adverse effects on individual viability and reproductive success (Alonso-Alvarez et al. 2004; Bize et al. 2008; Saino et al. 2011).

So far, one study only has tested if nestlings that are forced to beg more will suffer from higher oxidative stress levels (Moreno-Rueda et al. 2012). However, in this study, a single nestling was isolated from its siblings during the experiment without taking into consideration the dynamics of intersibling conflicts in the nest, which can strongly influence begging behavior, especially in species with many young born simultaneously (Smith and Montgomerie 1991; Rodríguez-Gironés et al. 1996; Mock and Parker 1997; Dreiss et al. 2010; Romano et al. 2012). Here, we manipulated begging behavior of great tit nestlings (*Parus major*), a species with relatively large broods with 8 offspring on average. We chose an experimental approach that did not affect overall feeding rate of parents but forced them to provide food from either 1 or 2 locations within the

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nest. As a result, nestlings had to compete more intensely for food at a single location. We could thus assess the oxidative cost of begging directly in the nest under competitive conditions. Several studies have shown that birds benefit from dietary antioxidants during early life stages (e.g., de Ayala et al. 2006; Hall et al. 2010; Marri and Richner 2014). One important antioxidant is vitamin E that scavenges free radicals and thus protects the individual from lipid peroxidation (Surai 2002). Vitamin E is assumed to alleviate the costs of begging by its antioxidant potential (Noguera et al. 2010). We supplemented half of each brood with vitamin E during the period of increased sibling competition to assess if vitamin E can compensate for the potentially negative effects of intense begging.

We aimed to test if elevated begging intensity leads to higher oxidative stress levels, different fledging probability, and/or differences in fitness-related traits of nestlings, such as body mass (e.g., Tinbergen and Boerlijst 1990).

We predicted that oxidative stress increases when nestlings are forced to beg more intensely. Furthermore, we expected that nestlings supplemented with vitamin E could afford to beg more without increased levels of oxidative stress.

## MATERIAL AND METHODS

### Experimental setup

The experiment was performed in spring 2012 in the forest Forst near Bern, Switzerland (46°7'N, 7°8'E), in a free-ranging population of great tits, breeding in artificial nest-boxes.

From 1 April onward, we visited nest-boxes regularly to determine laying and hatching dates. Hatching day was defined as day 0. On day 3 posthatch, all nestlings were weighed to the nearest 0.1 g and marked individually by selectively removing tuft feathers. On day 5, a sticker representing a dummy camera was placed on the top inside cover of the nest-box to accustom the birds to the presence of a real camera lens later.

In the morning of days 6 and 13 posthatching, we installed an infrared-sensitive camera in the upper part of each nest-box and video-recorded the nests for 2 h. Before filming, each nestling was marked with small spots of acrylic paint on the head to allow for individual recognition (Köl liker et al. 1998). The first 30 min of every video was excluded from all analyses to exclude potentially disturbing influences from setting up the camera. From video recordings of day 6 ( $n = 160$ ), we determined the feeding positions of male and female parent, using the different brightness of the head caps as indicator to distinguish the sexes. Feeding positions of great tit parents are strikingly consistent (Köl liker et al. 1998; Lessells et al. 2006) over the nestling period. The position was calculated as the angle between the head of the bird when feeding and the entrance hole. We identified nests in which male and female parent naturally fed from 2 different locations. This is a parental strategy to decrease competition among siblings because instead of competing for 1 site, nestlings can choose between 2 locations (Köl liker et al. 1998; Tanner et al. 2007). We calculated the median angle for each parent and excluded nests in which the 2 feeding locations of both parents were similar (angle < 90°) ( $n = 69$ ) and nests where only one parent entered the nest during the time of video recording. We then split the 84 remaining nests with 2 clearly distinct parental feeding locations (angle > 90°) in 2 groups: one group with a single feeding location and a second (control) group where nestlings could be fed from 2 opposed locations. We expected nestlings in the first group to show an increased

begging activity. We chose this method because sibling competition is manipulated in a way that does not affect the quantity of food delivered by the parents.

On day 8 posthatch, nestlings were ringed with aluminum rings, weighed, and their tarsus and wing length measured. We took a blood sample of 20 µL to determine the sex of each nestling and to measure oxidative stress levels. On the morning of the ninth day after hatching, nests were equipped with a horizontal barrier between the nestlings and the parents. This barrier, a mesh with a Plexiglas plate in the middle, was placed 5 cm above the nest cup and had either 1 or 2 openings through which the parents were able to feed their nestlings (for a detailed description of the method, see Tanner et al. 2007).

Nests were randomly assigned to either the treatment with 1 feeding location and hence higher nestling competition for access to food (begging treatment) or the control treatment with 2 feeding locations. In 7 nests with 1 opening and 2 nests with 2 openings, all nestlings died at the beginning of the experimental phase, and we replaced a failed nest immediately with another one of the same treatment group in order to get a balanced final sample size on day 13 of 37 nests (224 nestlings) in the begging treatment and 38 nests (230 nestlings) in the control treatment.

Since the spring of 2012 was very harsh with temperatures below 0° during the night until the middle of May, we removed the barriers in the evening between 8 and 9 PM to allow the female parent to sleep in the nest-box with offspring and replaced the barrier early in the morning between 6 and 7 AM just after parents resumed feeding activities. The sequence of placement and displacement was alternated among nests every day. The barrier was removed permanently after the video recording on day 13, and nestlings were weighed, measured, and blood sampled again. The blood samples were centrifuged, and the plasma stored at -20 °C until used for analyzing oxidative stress levels. From day 17 onward, nests were visited daily to determine the fledging day and identity of the fledged nestlings.

### Vitamin E supplementation

We aimed to double the daily intake of vitamin E between the periods of increased competition. Therefore, we supplemented half of each brood with  $\alpha$ -tocopherol, which has the greatest antioxidant potential of the 8 tocopherol and tocotrienol derivatives that vitamin E comprises (Surai 2002). On day 8, nestlings were ranked according to body mass. In each nest, the first nestling was randomly assigned to one level of the vitamin treatment by tossing a coin, and the treatment was then alternated between odd and even nestlings.

The quantity of vitamin E was calculated according to the estimated daily food intake (DFI) of great tits reported by Crocker et al. (2002), that is, 22.15 g of caterpillars, the main food source of great tits (Gosler 1993). A surplus for fast-growing nestlings was taken into account (de Ayala et al. 2006). The concentration of vitamin E in the daily diet was calculated by using the weighted mean between the quantities reported by Catoni et al. (2008) and Arnold et al. (2010), that is, 24.4 µg/g. The multiplication of the DFI by the concentration of vitamin E in the food resulted in a supplemented dose of 0.49 µg of  $\alpha$ -tocopherol acetate (Sigma-Aldrich, Basel, Switzerland) per day. Each nestling received twice (on days 9 and 11) 1 *Calliphora* spp. larva coated with the double of the daily dose of the vitamin they obtain from their natural diet between days 9 and 12. Control nestlings received a plain larva. We chose this relatively low dose of vitamin E because we aimed to stay within the natural range of vitamin E intake of great tits

because high doses of the vitamin might lead to the absence of a positive effect (de Ayala et al. 2006) or potentially to opposing effects (Surai 2002).

### Begging intensity and provisioning rate per nestling

From the videos, we determined during 1.5 h the provisioning rate of each nestling and the mean size of prey items received. We analyzed in total 84 nests on day 6 and 66 nests (33 per begging treatment) on day 13.

To assess begging behavior, we used begging intensity as a proxy for all aspects of begging of great tit nestlings because different begging features, including nestling mobility, are highly correlated in this species (Neuenschwander et al. 2003). Nestling begging intensity was measured for 1 h on a 5-level scale: 0 = calm, 1 = weak gaping, 2 = persistent gaping, 3 = gaping and neck fully stretched, and 4 = gaping, neck fully stretched, and wing flapping (Köllicker et al. 1998) for every feeding bout. We calculated a mean begging score for each individual by dividing the begging scores by the number of visits (for 597 nestlings from 83 nests on day 6 and for a subsample of 292 nestlings from 50 nests on day 13).

### Antioxidant capacity

Nestling antioxidant capacity was determined using KRL (Kit Radicaux Libres®) test (Brevet Spiral, V02023, Couternon, France) adapted to bird physiological parameters (Alonso-Alvarez et al. 2004). This assay measures whole blood resistance to oxidative stress by assessing the time required to hemolyze 50% of red blood cells of the sample when exposed to a free-radical attack. Seven microliters of the whole blood was diluted into 255.5  $\mu$ L of KRL buffer (150mM Na<sup>+</sup>, 120mM Cl<sup>-</sup>, 6mM K<sup>+</sup>, 24mM HCO<sub>3</sub><sup>-</sup>, 2mM Ca<sup>2+</sup>, 340 mOsm, pH 7.4) immediately after sampling and stored at 4 °C before analysis within 10h after blood collection. We pipetted 80  $\mu$ L of KRL-diluted blood into a 96-well microplate and added in each well 136  $\mu$ L of a 150mM solution of 2,2-azobis-(amidinopropane) hydrochloride, a free-radical generator. The microplate was incubated and read at 40 °C with a microplate reader spectrophotometer (PowerWave XS reader, Witec AG, Switzerland). The rate of hemolysis was assessed by the change in optical density at 540nm; the initial optical density was used as an estimation of the hematocrit, which is likely to influence the rate of hemolysis. Readings were conducted every 3.5 min for a total duration of 80 min.

The repeatability of the method, evaluated by using samples from great tits that were not included in this study, was high ( $r = 0.78$ ,  $P < 0.001$ ,  $n = 80$ ).

### Oxidative damage

The plasma concentrations of malondialdehyde (MDA), caused by the  $\beta$ -scission of peroxidized fatty acids, were measured by using high-performance liquid chromatography (HPLC) with fluorescence to assess oxidative damage. This method was already successfully used in many ecological studies (e.g., Losdat et al. 2013; Marri and Richner 2014).

Chemical solutions were prepared using ultrapure water (Milli-Q Synthesis; Millipore, Watford, UK). We used 2-mL screw-top microcentrifuge tubes to derivatize samples. Five microliters of sample or standard (1,1,3,3-tetraethoxypropane, TEP), 5  $\mu$ L of butylated hydroxytoluene solution (0.05% w/v in 95% ethanol), 40  $\mu$ L of phosphoric acid solution (0.44M), and 10  $\mu$ L of thiobarbituric acid (TBA) solution (42mM) were pipetted to the tube and

vortexed for 5 s. Then the samples were incubated at 100 °C for 1 h in a dry bath incubator to allow the formation of MDA–TBA adducts. Subsequently, samples were cooled on ice for 5 min, and after this, 80  $\mu$ L of *n*-butanol was added to each tube and samples were vortexed for 20 s. Tubes were then centrifuged for 4 min at 4 °C and 13 000 rpm to separate the 2 phases, and 55  $\mu$ L of the upper phase was transferred to an HPLC vial for analysis.

Samples (40  $\mu$ L) were injected into a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA) fitted with a Hewlett-Packard Hypersil 5  $\mu$ m ODS 100 $\times$ 4.6mm column and a 5  $\mu$ m ODS guard column maintained at 37 °C. The mobile phase was methanol buffer (40:60, v/v), consisting of a 50mM anhydrous solution of potassium monobasic phosphate at pH 6.8 (adjusted using 5M potassium hydroxide solution), running isocratically over 3.5 min at a flow rate of 1 mL/min. Data were collected using a fluorescence detector (RF2000; Dionex) at 515 nm (excitation) and 553 nm (emission). The standard curve was prepared using a TEP stock solution (5  $\mu$ M in 40% ethanol) serially diluted using 40% ethanol for calibration. This method was highly repeatable ( $r = 0.87$ ,  $P < 0.0001$ ,  $n = 80$ ).

### Statistical procedures

Data were analyzed with R version 2.15.1 (R Development Core Team 2010), using nlme and lme4 library.

First, we checked whether the experimental treatment groups were randomized with respect to begging intensity and oxidative stress values before the experiment started, by using linear mixed-effect models with restricted maximum likelihood and a generalized linear mixed model with Poisson distribution for the feeding rate on day 6. We checked the randomization of brood size and hatching day between begging treatments with general linear models with normal error distribution.

To analyze begging intensity, oxidative stress values, and nestling body mass on day 13, we used linear mixed-effect models with restricted maximum likelihood.

Explanatory variables in all initial models were begging treatment, vitamin treatment, brood size on day 8, hatching day, and nestling sex. In the model of body mass on day 13, we included body mass on day 8 as a covariate to account for possible differences before the experiment started. The mass-based rank of the nestling on day 8 was included in the models for begging intensity, oxidative damage, and antioxidant capacity. In the model of begging intensity, we replaced brood size on day 8 with brood size on day 13 because the number of attendant siblings can strongly influence begging intensity. We included the initial optical density as a covariate in the model for antioxidant capacity (KRL). Two-way interactions between the 2 treatments and each treatment with nestling sex were included in all models.

To assess whether our treatments did not induce a change in the individual provisioning rate or mean prey size per nestling on day 13, a generalized linear mixed model with Poisson distribution of errors and a linear mixed-effect model were used. Both models included the same explanatory variables as mentioned before, and we additionally controlled for the response variable of the other model, respectively.

The individual fledging probability was analyzed with a generalized linear mixed model with binomial error distribution, with the same variables as above and body mass on day 13 as additional explanatory variable, because body mass is a strong predictor for nestling survival and it was not influenced by the treatments itself (see Results).

We used a generalized linear mixed model with binomial error distribution to assess differences in mortality between the 2 begging treatments during the experimental phase. The begging treatment, sex, and their interaction were included as fixed factors, and hatching date and brood size on day 8 as covariates.

Nest of origin was included in all mixed models as a random effect to account for nonindependence of nestlings raised in the same nest. Nonsignificant terms were backward eliminated using maximum likelihood estimation starting with the exclusion of interactions. We checked the fit of the models by plotting residuals against fitted values and by inspecting residuals for normality and homoscedasticity.

RESULTS

Validation of experimental setup

Before the experiment started, treatment groups did not differ with regard to begging intensity (begging treatment:  $F_{1,80} = 1.79$ ,  $P = 0.19$ ; vitamin treatment:  $F_{1,470} = 1.96$ ,  $P = 0.16$ ) and feeding rates (begging treatment:  $z = 1.58$ ,  $P = 0.12$ ; vitamin treatment:  $z = 0.66$ ,  $P = 0.96$ ). Oxidative stress levels also showed no significant difference between experimental groups (oxidative damage [MDA]—begging treatment:  $F_{1,76} = 0.21$ ,  $P = 0.65$ ; vitamin treatment:  $F_{1,175} = 0.08$ ,  $P = 0.77$ ; antioxidant capacity [KRL]—begging treatment:  $F_{1,79} = 0.16$ ,  $P = 0.69$ ; vitamin treatment:  $F_{1,252} = 0.22$ ,  $P = 0.63$ ).

The begging treatment was also balanced with respect to brood size on day 8 (GLM:  $F_{1,82} = 0.35$ ,  $P = 0.55$ ) and hatching day ( $F_{1,82} = 0.039$ ,  $P = 0.85$ ).

Begging intensity

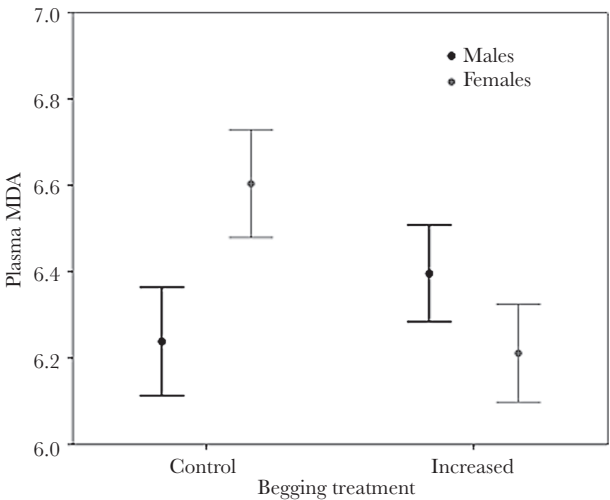
Nestlings in nests where parents were experimentally forced to feed from one location begged more intensely and male nestlings begged more than their female siblings (Table 1). Begging intensity increased with hatching date, and nestlings in smaller broods begged more intensely (Table 1). The vitamin E supplementation did not influence the begging intensity, neither did the interaction

between treatments nor the interactions between treatments and sex (Table 1).

Oxidative stress measurements

Nestlings supplemented with vitamin E tended to have lower MDA levels (Figure 1), and MDA levels showed a decrease over the breeding season (Table 2). MDA was significantly influenced by the interaction between the begging treatment and sex (Figure 2). The MDA levels of males did not differ between treatment groups, whereas females had a lower MDA in the increased begging group (post hoc analysis—males:  $F_{1,66} = 0.125$ ,  $P = 0.41$ ; females:  $F_{1,62} = -0.41$ ,  $P = 0.024$ ).

In the control begging group, oxidative damage was higher in females than in males, whereas in the increased begging group, there was no difference between sexes (post hoc analysis—control:  $F_{1,116} = 10.18$ ,  $P = 0.0018$ ; increased:  $F_{1,122} = 1.99$ ,  $P = 0.16$ ).



**Figure 1** Concentration of plasma MDA ( $\mu\text{mol/L}$ ) on day 13 posthatch for males and females in relation to the begging treatment. Mean values are shown with standard errors.

**Table 1**

Linear mixed-effect model testing the effect of the vitamin E treatment and the begging treatment on begging intensity on day 13 after hatching

Variables	Estimate $\pm$ SE	F	df	P
Intercept	1.59 $\pm$ 0.81			
<b>Hatching day</b>	<b>0.02 <math>\pm</math> 0.01</b>	<b>4.76</b>	<b>1,46</b>	<b>0.034</b>
<b>Brood size day 13</b>	<b>-0.17 <math>\pm</math> 0.05</b>	<b>10.36</b>	<b>1,46</b>	<b>0.002</b>
Rank day 8	-0.01 $\pm$ 0.02	0.11	1,210	0.746
<b>Vitamin treatment<sup>a</sup></b>	<b>0.07 <math>\pm</math> 0.07</b>	<b>0.93</b>	<b>1,211</b>	<b>0.336</b>
<b>Begging treatment<sup>b</sup></b>	<b>0.22 <math>\pm</math> 0.11</b>	<b>4.22</b>	<b>1,46</b>	<b>0.046</b>
<b>Sex<sup>c</sup></b>	<b>0.20 <math>\pm</math> 0.08</b>	<b>6.94</b>	<b>1,211</b>	<b>0.009</b>
Vitamin treatment $\times$ sex	0.26 $\pm$ 0.15	2.98	1,209	0.086
Begging treatment $\times$ sex	-0.04 $\pm$ 0.16	0.07	1,207	0.792
Begging treatment $\times$ vitamin treatment	0.16 $\pm$ 0.15	1.12	1,208	0.291

F and P values of nonsignificant terms are those just before removal from the model. Nest was included in the model as a random factor. Terms of the final model are highlighted in bold. df, degrees of freedom; SE, standard error.

<sup>a</sup>Relative to nestlings not supplemented with vitamin E.

<sup>b</sup>Relative to control nestlings.

<sup>c</sup>Relative to female nestlings.

**Table 2**

Linear mixed-effect model testing the effect of the vitamin E treatment and the begging treatment on oxidative damage (MDA) on day 13 after hatching

Variables	Estimate $\pm$ SE	F	df	P
Intercept	9.58 $\pm$ 0.67			
<b>Hatching day</b>	<b>-0.04 <math>\pm</math> 0.01</b>	<b>20.04</b>	<b>1,71</b>	<b>&lt;0.0001</b>
Brood size day 8	0.04 $\pm$ 0.05	0.41	1,70	0.526
Rank day 8	-0.01 $\pm$ 0.02	0.35	1,238	0.556
<b>Vitamin treatment<sup>a</sup></b>	<b>-0.14 <math>\pm</math> 0.08</b>	<b>3.37</b>	<b>1,239</b>	<b>0.0678</b>
<b>Begging treatment<sup>b</sup></b>	<b>-0.39 <math>\pm</math> 0.15</b>	<b>7.12</b>	<b>1,71</b>	<b>0.0094</b>
<b>Sex<sup>c</sup></b>	<b>-0.37 <math>\pm</math> 0.11</b>	<b>10.21</b>	<b>1,239</b>	<b>0.0016</b>
Vitamin treatment $\times$ sex	0.022 $\pm$ 0.16	0.02	1,236	0.892
<b>Begging treatment <math>\times</math> sex</b>	<b>0.55 <math>\pm</math> 0.16</b>	<b>11.39</b>	<b>1,71</b>	<b>0.0009</b>
Begging treatment $\times$ vitamin treatment	0.82 $\pm$ 0.16	0.28	1,237	0.560

F and P values of nonsignificant terms are those just before removal from the model. Nest was included in the model as a random factor. Terms of the final model are highlighted in bold.

<sup>a</sup>Relative to nestlings not supplemented with vitamin E.

<sup>b</sup>Relative to control nestlings.

<sup>c</sup>Relative to female nestlings.



There was no significant interaction effect between the vitamin treatment and sex on MDA levels or between treatments (Table 2).

The antioxidant capacity (KRL) was not influenced by the treatments (begging treatment:  $F_{1,72} = 0.85$ ,  $P = 0.36$ ; vitamin treatment:  $F_{1,266} = 2.85$ ,  $P = 0.093$ ) and positively correlated with the initial optical density (estimate:  $0.14 \pm 0.06$ ,  $F_{1,266} = 6.12$ ,  $P = 0.01$ ). None of the other variables or interaction terms influenced antioxidant capacity significantly (all  $P > 0.1$ ).

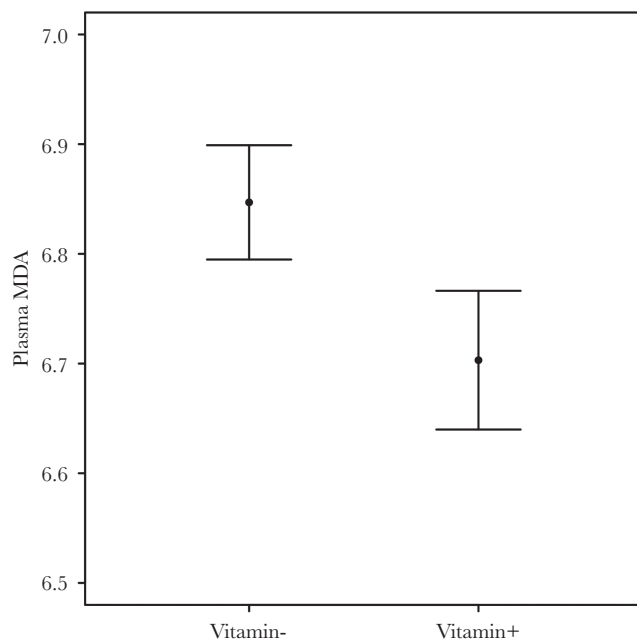
### Provisioning rate per nestling and prey size

The number of feeding visits per nestling did not differ between treatment groups (begging treatment:  $z = -0.93$ ,  $P = 0.35$ ; vitamin treatment:  $z = 0.17$ ,  $P = 0.86$ ) but was negatively correlated with the size of prey items it received (estimate:  $-0.38 \pm 0.07$ ,  $z = -5.21$ ,  $P < 0.001$ ) and the rank of the nestling on day 8 (estimate:  $-0.02 \pm 0.01$ ,  $z = -2.52$ ,  $P = 0.01$ ).

Prey size was independent of both treatments (begging treatment:  $F_{1,62} = 0.05$ ,  $P = 0.50$ ; vitamin treatment:  $F_{1,313} = -0.02$ ,  $P = 0.64$ ). Nestlings born later in the season received on average bigger prey items (estimate:  $0.01 \pm 0.001$ ,  $F_{1,62} = 4.68$ ,  $P = 0.03$ ). Neither individual provisioning rate nor prey size was linked to any other variable or interactions (all  $P > 0.1$ ).

### Body mass

Body mass on day 13, corrected for body mass on day 8, did not differ between treatment groups (begging treatment:  $F_{1,71} = 0.28$ ,  $P = 0.60$ ; vitamin treatment:  $F_{1,329} = 0.01$ ,  $P = 0.99$ ). Nestlings born later in the season were significantly lighter (estimate:  $-0.05 \pm 0.02$ ,  $F_{1,71} = 4.65$ ,  $P = 0.03$ ) and nestlings that were raised in a nest with a larger brood size tended to be lighter (estimate:  $-0.27 \pm 0.14$ ,  $F_{1,71} = 3.6$ ,  $P = 0.06$ ). Male nestlings were heavier than their female siblings (estimate:  $0.36 \pm 0.12$ ,  $F_{1,329} = 3.6$ ,  $P = 0.01$ ). There was no effect of any of the interaction terms on body mass (all  $P > 0.1$ ).



**Figure 2**

Concentration of plasma MDA ( $\mu\text{mol/L}$ ) on day 13 posthatch in relation to the vitamin treatment. Mean values are shown with standard errors.

### Fledging probability

Individual fledging probability was higher for nestlings supplemented with vitamin E (Table 3 and Supplementary Appendix) and for nestlings with a higher body mass on day 13 (Table 3). Nestling sex, hatching day, treatment interaction, or the interaction of each treatment with sex did not affect fledging probability (Table 3). Fledging probability showed a weak nonsignificant negative correlation with brood size on day 8 (Table 3).

### Mortality during experimental phase

Nestling mortality between day 8 and day 13 was not affected by the begging treatment ( $z = 1.48$ ,  $P = 0.14$ ) or the interaction between begging treatment and sex ( $z = -0.33$ ,  $P = 0.74$ ). Mortality in this period tended to be positively correlated with hatching date ( $z = 1.75$ ,  $P = 0.08$ ), and there was a weak tendency for males to have lower mortality ( $z = -1.73$ ,  $P = 0.08$ ). None of the other variables affected mortality between days 8 and 13 (all  $P > 0.1$ ).

## DISCUSSION

In the present study, we experimentally increased begging intensity of great tit nestlings to test if begging rises oxidative stress. Begging intensity was indeed increased in the experimental group, whereas the provisioning rate and the size of delivered food items were not significantly affected as intended.

Experimentally increased begging had differential effects on oxidative damage in the 2 sexes. In the control group, male nestlings had a significantly lower oxidative damage than females. This is consistent with the results of another study in the same species (Losdat et al. 2014) and of a study in decorated crickets (*Gryllodes sigillatus*) (Archer et al. 2013, but see Markó et al. 2011; *Ficedula albicollis*).

However, when competition was experimentally increased, a difference in oxidative stress values between sexes was not detectable anymore due to the fact that female nestlings had significantly less oxidative damage than those in the control nests, whereas male oxidative damage levels remained stable. This result suggests that strong competition may trigger females to invest more into the resistance to oxidative stress. This is puzzling, however, because

**Table 3**

**Individual fledging probability in relation to the begging and vitamin E treatment, using a binomial generalized linear mixed model**

Variables	Estimate $\pm$ SE	$z$	$P$
Intercept	$-14.8 \pm 2.04$		
Hatching day	$-0.04 \pm 0.06$	$-0.74$	0.458
Brood size day 8	$-0.64 \pm 0.37$	1.08	0.079
<b>Mass 13</b>	<b><math>1.28 \pm 0.16</math></b>	<b>8.28</b>	<b>&lt;0.0001</b>
<b>Vitamin treatment<sup>a</sup></b>	<b><math>0.78 \pm 0.37</math></b>	<b>2.12</b>	<b>0.034</b>
<b>Begging treatment<sup>b</sup></b>	<b><math>0.66 \pm 0.77</math></b>	<b>0.87</b>	<b>0.387</b>
Sex <sup>c</sup>	$-0.03 \pm 0.42$	$-0.07$	0.943
Vitamin treatment $\times$ sex	$0.18 \pm 0.73$	0.25	0.807
Begging treatment $\times$ sex	$0.79 \pm 0.36$	$-0.71$	0.476
Begging treatment $\times$ vitamin treatment	$0.36 \pm 0.71$	0.51	0.608

Nest was included in the model as a random factor. Terms of the final model are highlighted in bold.

<sup>a</sup>Relative to nestlings not supplemented with vitamin E.

<sup>b</sup>Relative to control nestlings.

<sup>c</sup>Relative to female nestlings.

female great tit nestlings usually suffer more from competition than males (Oddie 2000), and thus, we would expect the opposite effect.

Nestlings, especially females in the increased begging group, may have reduced investment in other antioxidant demanding traits, for example, their immunocompetence, to lower oxidative damage. We did not test here the effect of the treatments on the immune response and can, therefore, not exclude that this kind of trade-off arose.

Moreno-Rueda et al. (2012) found an effect of intense begging on oxidative stress in magpie (*Pica pica*) nestlings, but only when controlling for growth and immune response. Therefore, they suggested that individuals can reduce growth and immune response in favor of a better oxidative status. Possible sex-specific differences in the reaction to increased begging were not assessed in their study.

A study by Moreno-Rueda (2010) on house sparrows (*Passer domesticus*) also supports the suggestion that nestlings may respond to enforced high begging levels by downregulating their immune system, based on the finding that house sparrow (*P. domesticus*) nestlings showed depressed immunocompetence when forced to beg for a longer time period. However, this does not explain why males and females did not respond similarly to the begging treatment. Our result suggests that females and males are likely follow unequal strategies in the allocation of limited resources when competition is increased. Future studies should take this into account.

As another possibility, increased scramble competition may have stronger sublethal effects in a breeding season with severe environmental conditions, as in the year of our study, because more competitive nestlings may have monopolized the scarce resources (Royle et al. 2012). Although not statistically significant ( $P = 0.16$ , Fisher's Exact test), a few more broods died in the increased begging treatment before the age of 13 days (7 vs. 2). Furthermore, males tended to have better survival during the experimental phase, independent of the begging treatment. We cannot fully exclude that this could have partly masked effects of our treatments, due to the fact that lower quality individuals may have died in the begging treatment before the final blood sample could be taken and begging levels assessed. The probability to fledge and antioxidant capacity were not influenced by the begging treatment or by sex.

We could not detect any growth cost of begging. In general, evidence for a cost of begging via impaired growth rates is rather contradictory and seems to differ among species. Some studies observed an effect of begging intensity on growth rates (Kilner 2001; Rodriguez-Girones et al. 2001), but this effect was absent in other studies (Kedar et al. 2000; Leonard et al. 2003; Moreno-Rueda 2010). The costs of begging may differ among species or depend on environmental conditions, and their effect on growth thus remains intriguing.

Furthermore, we raised the availability of vitamin E during the period of increased begging. We expected an enhancing effect of vitamin E on begging intensity if begging is an antioxidant demanding behavior. Against this prediction, begging was not affected by vitamin E intake, which is in contrast to a previous study that found a positive effect of vitamin E on a specific type of begging in yellow-legged gulls (*Larus michahellis*) (Noguera et al. 2010). However, oxidative stress was not measured here, so, and thus, it is not clear if this increase in begging calls was mediated by oxidative stress levels.

A higher availability of vitamin E increased the fledging probability of nestlings in our study. The positive effect of vitamin E on fledging probability may be explained by its tendency to decrease oxidative damage. This is supported by the fact that

oxidative damage on day 13 was correlated with fledging probability ( $\chi^2 = 4.68$ ;  $P = 0.0305$ , estimate  $\pm$  standard error:  $0.72 \pm 0.33$ ). The relationship between oxidative damage and fledging probability has not been demonstrated in great tit nestlings to date (Losdat et al. 2013), but it can be assumed that low oxidative damage at an early age is an important factor for offspring survival before and after fledging. In another species, it has been shown to influence recruitment probability (Noguera et al. 2012). A positive effect of vitamin supplementation on fledging success was also found in a previous study (Marri and Richner 2014) on great tits although in that study, mass gain also showed an increase in the supplemented group. Mass gain was not influenced by the supplement in our study and, hence, suggests that the effect of vitamin E on fledging success does not need to be mediated by an increase in body mass.

The supplementation with vitamin E did not influence the antioxidant capacity in the plasma. Vitamin E levels in the plasma were not measured. Because vitamin E is a lipophilic vitamin and often stored in other tissues, for example, the liver (Surai 2002), the levels circulating in the blood do not necessarily reflect the quantity of absorbed vitamin. Larcombe et al. (2010) found no increase in levels of  $\alpha$ -tocopherol in the plasma of blue tits (*Cyanistes caeruleus*) after supplementation, and de Ayala et al. (2006) reported a dose-independent effect of vitamin E supplementation on plasma levels.

It is unlikely that the supplementation was ineffective given first that the same method was already used successfully in other studies (Losdat et al. 2011; Marri and Richner 2014) and second that the supplementation had a positive effect on fledging probability. Alternatively, other properties of vitamin E rather than its antioxidant potential could have affected nestling survival until fledging, for example, its beneficial effects on the immune system (Surai 2002).

In conclusion, an oxidative cost of begging could not be detected in this study, and our data do not support an enhancing effect of vitamin E on begging intensity. However, vitamin E influenced fledging probability and thus short-term survival positively.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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